American Journal of Hematology 63:184–191 (2000)

A Polymorphism of the X-Linked Gene IDS Increases the Number of Females Informative for Transcriptional Clonality Assays

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Studies of clonality have been essential for understanding the hierarchy of hematopoiesis and the biology of malignancies. Most clonality assays are based on the X chromosome inactivation phenomenon in females; these assays detect protein polymorphisms, differences in DNA methylation, or transcripts of the active X chromosome. Assays based on protein polymorphisms or DNA methylation have significant shortcomings. The major disadvantage of transcriptional assays is their limited applicability since only approximately half of females are informative for these studies. We have developed a new transcriptional assay based on an exonic polymorphism of the X-chromosome gene IDS. This gene is located in the same X-chromosome region (Xq28) as G6PD and p55, two genes with exonic polymorphisms for which we previously developed transcriptional assays. We developed non-radioactive PCR-based assays for rapid screening of genotype and determination of clonality. We also report reaction conditions for a quantitative ligase detection assay of IDS allelic transcripts. The frequency of the IDS polymorphism is 46% in Caucasian females and 39% in African-American females; in combination with G6PD and p55, 76% of Caucasian females and 62% of African-American females are informative for these assays. While this gene is highly polymorphic in Caucasian and African-American females, it is not informative in Oriental females. We established that the IDS gene is in linkage equilibrium with G6PD and p55. Unlike methylation-based assays, this assay is suitable for studying clonality in non-nucleated cells such as platelets and reticulocytes. With the discovery of exonic polymorphisms of other X-chromosome genes, all females should eventually be suitable for X-chromosome transcriptional clonality analysis. Am. J. Hematol. 63:184–191, 2000. © 2000 Wiley-Liss, Inc.

Key words: X-chromosome inactivation; clonality assay; hematopoiesis; polymorphism

INTRODUCTION

The ability to identify monoclonal cell populations is useful for diagnosing malignancy, following malignant disease progression and remission, and studying normal and abnormal hematopoiesis. Many methods of determining clonality, including analysis of viral integration and detection of characteristic somatic gene rearrangements, translocations, deletions, and point mutations, are limited by their applicability to only certain tumors or cell types [1]. In contrast, clonality assays based on the phenomenon of X-chromosome inactivation, although restricted to informative females, are widely applicable and serve as an independent marker of clonality.

According to the Lyon–Beutler hypothesis of random X-chromosome inactivation [2–4], one of the two X chromosomes, either the maternally derived (Xm) or the paternally derived (Xp), is inactivated in every female somatic cell early in embryogenesis. The same pattern of X inactivation is maintained in the progeny of the cells [5]. Thus, normal female tissues have a mosaic expression of genes from both Xp and Xm, but cells from a monoclonal population express genes from only Xp or Xm. In X-inactivation based clonality assays, Xm and Xp are first distinguished by the presence of polymorphisms of X-linked genes; in females who are heterozy-

Contract grant sponsor: Leukemia Society of America; Contract grant number: 6151-98.

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Received for publication 10 May 1999; Accepted 3 November 1999

gous for the polymorphism, identification of the active X chromosome(s) by gene expression at the protein or mRNA level or by differences in DNA methylation determines clonality.

Electrophoretic differences between glucose-6-phosphate dehydrogenase (G6PD) isozymes were first used to establish a clonal origin for a variety of tumors [6–8] but the relatively few numbers of heterozygous females limited the overall usefulness of this method [9]. Subsequent clonality assays used methylation-sensitive restriction endonucleases to detect DNA methylation pattern differences between active and inactive X-chromosome-linked polymorphic loci such as the hypoxanthine phosphoribosyltransferase gene [10], the phosphoglycerate kinase gene [11,12], the human androgen receptor gene [13], and DXS255 (M27β probe) [14,15]. Although the number of heterozygous females is substantially increased by these assays, the heterogeneity of methylation patterns between active and inactive X-chromosomes [16] and the influence of other factors such as hematological malignancy on methylation patterns [17,18] complicates interpretation of these assays. In addition, DNA methylation analysis is not applicable to non-nucleated cells such as platelets and reticulocytes.

We previously developed clonality assays that identify the active X chromosome by gene expression at the mRNA level [19–22]. These assays detect silent exonic polymorphisms of two ubiquitously expressed X-chromosome genes, G6PD (C/T at cDNA #1311) and palmitoylated membrane protein p55 (G/T at cDNA #358). The original assay used reverse transcription polymerase chain reaction followed by ligase detection reaction (rtPCR-LDR) to detect the polymorphism (Fig. 1). LDR is based on the ability of DNA ligase to covalently link two oligonucleotide probes that are perfectly base-paired to the target; thus, LDR can identify a single nucleotide substitution.

Although specific and quantitative, rtPCR-LDR is time-consuming and requires exposure to radioactive material. As a simpler screening method for detecting the polymorphisms, we developed another assay, allele specific polymerase chain reaction, or ASPCR [22], which is based on the previously reported techniques of allele-specific oligonucleotide hybridization (ASOH) [23], PCR-ASOH [24], and PCR amplification of a specific allele (PASA) [25]. ASPCR consists of two rounds of semi-nested PCR (Fig. 2). In the first round, the region of DNA containing the polymorphism is amplified. The allele specific round is performed in two tubes that both contain first round products and a common reverse primer but different, allele-specific, forward primers. ASPCR provides a rapid, non-radioactive method of screening genomic DNA for genotype and cDNA for clonality. Nevertheless, a limitation of these assays for the G6PD and p55 transcriptional polymorphisms is that

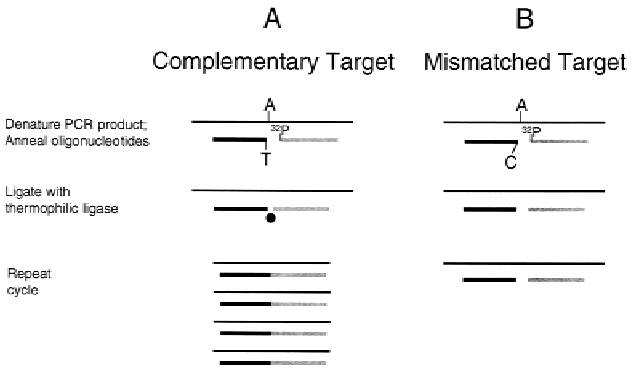

Fig. 1. Ligase detection reaction. In this reaction, one of the two upstream oligonucleotide probes differing only in the 3′ nucleotide and a common downstream probe are annealed to a PCR product containing the polymorphic region. The 3′ end of the upstream probe is immediately adjacent to the 5′ end of a downstream probe labeled with ^{32}P. A non-specific tag (e.g., TA) is added to the 5′ end of one of the upstream probes, permitting differentiation of the allelic products by size on a polyacrylamide denaturing gel. (A) Nucleotides are perfectly matched to the target, allowing DNA thermophilic ligase to covalently link the two oligonucleotides. Repeating the cycle linearly increases the product. (B) Upstream probe is mismatched at the 3′ nucleotide; ligation does not occur.

only 50% of females are heterozygous and thus suitable for clonality analysis [21].

Another silent exonic polymorphism, a C to T at the third nucleotide of codon 146, has been reported in the iduronate-2-sulfatase (IDS) gene [26,27]. The IDS gene is located on Xq27.3-28 [28,29] and encodes a lysosomal enzyme. To increase the number of females informative for clonality studies, we developed an assay based on the detection of this polymorphism of the IDS gene. We also report the gene frequencies of this polymorphism in three major US ethnic groups and linkage analysis of the three closely located X-chromosome genes G6PD, p55, and IDS.

MATERIALS AND METHODS

Subjects

For genotype determination, peripheral blood samples from randomly selected individuals of three defined racial groups, Caucasian, African-American, and Oriental, were obtained. For clonality determination, peripheral blood samples from individuals with diagnosed or suspected myeloproliferative disorders were obtained.

Isolation of Blood Cells

EDTA anti-coagulated peripheral blood was obtained for preparation of myeloid cells and heparinized blood was used for mononuclear cell preparation. Neutrophils, reticulocytes, and platelets were separated by differential centrifugation, isopyknic density gradient separation, and

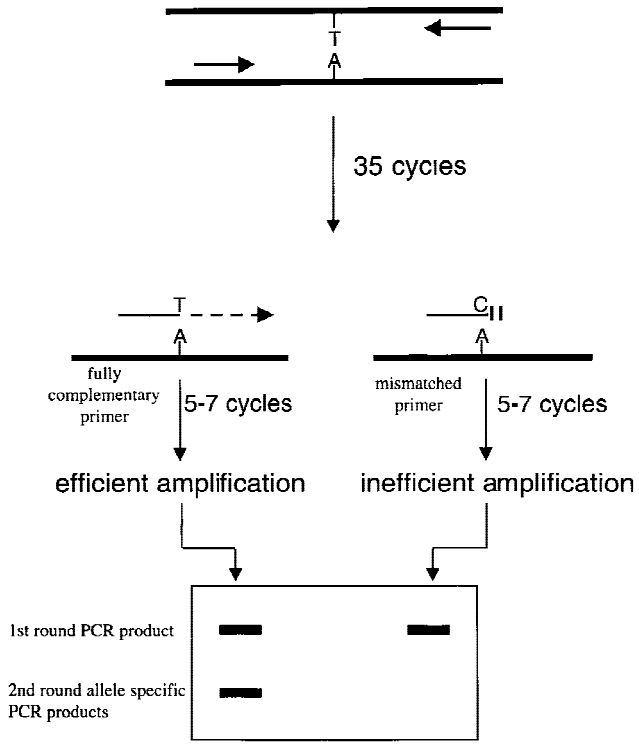

Fig. 2. Allelespecific PCR. The first round of PCR amplifies the region containing the single base polymorphism. The second, semi-nested, round of allele specific PCR is performed in two separate reaction tubes. These reactions differ only in the upstream primer used. The upstream primers are identical except at their 3′ ends: one is fully complementary to one allele, the other is complementary to the other allele. Efficient amplification occurs only in the reaction with the matched primer. The products from both reactions are analyzed simultaneously on agarose gel, permitting identification of the allele present.

dextran sedimentation using standard protocols [30]. Mononuclear cells isolated from Histopaque-1077 (Sigma, St. Louis, MO) gradient were washed in phosphate buffered saline (PBS; Sigma, St. Louis, MO) and labeled with the appropriate antibodies (Becton Dickinson, Mountain View, CA). The labeled cells were further purified into CD3+ T lymphocytes, CD19+ B lymphocytes, CD56+ NK cells, and CD14+ monocytes using a FACS Star Plus® cell sorter (Becton Dickinson, Mountain View, CA).

DNA Preparation

Red blood cells from whole blood were lysed in NH_4HCO_3/NH_4CI; the sedimented leukocytes were digested with proteinase K (2 mg/mL) in a buffer containing 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1% (w/v) SDS at 37°C overnight. Impurities were removed by the salting-out method with saturated sodium chloride [31]. DNA was precipitated with 2 volumes of 100% ethanol and washed in 70% ethanol. After a brief air-drying, the DNA pellet was dissolved in 10 mM Tris-HCl (pH 8.0) and stored at 4°C until analysis.

RNA Preparation

Reticulocyte RNA was prepared by acid precipitation from NH_4HCO_3/NH_4Cl hemolysate as described elsewhere [32]. This acid precipitate and cell lysates from other cells were used for RNA isolation using RNA-STAT (Tel-Test "B", Friendswood, TX) according to the manufacturer's recommendations. RNA samples were dissolved in DEPC-treated water and stored at −80°C.

Reverse Transcription of RNA

cDNA was synthesized using SuperScript II reverse transcriptase (Life Technologies, Grand Island, NY) with 1 μg/reaction random hexanucleotide primers (Pharmacia Biotech, Uppsala, Sweden). The reaction conditions for first-strand cDNA followed the manufacturer's instructions for transcription from less than 1 μg of total RNA.

Analysis of Genomic DNA for Genotype Determination by ASPCR

ASPCR for G6PD and p55 genotype was performed as previously described [22]. For determination of the IDS genotype, the region of DNA containing the polymorphism was amplified using forward primer IDS1 and reverse primer IDS2R. (Primer sequences are shown in Table I: primer location is illustrated in Fig. 3.) Fifteen picomoles of each primer and 1 unit of Taq DNA polymerase (Life Technologies, Grand Island, NY) were added to a 50 μL PCR reaction tube containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2 mM $MgCl_2$, and 300 μM dNTP. Thirty-five cycles were performed in a GeneAmp PCR System 9600 (Perkin-Elmer, Emeryville, CA) with the following parameters: 40 sec at 94°C; 30 sec at 48°C; and 30 sec at 72°C. Aliquots of 10 μL of products from each reaction were analyzed directly on a 1% agarose gel with 0.5 μg/mL ethidium bromide.

The second, allele-specific, round is a semi-nested PCR in which 5 μL of the first round products, 1 unit of Taq polymerase, 15 pmol of the common reverse primer IDS2R, and 15 pmol of an allele-specific primer, either IDS3C or IDS4T, were added to a 50 μL PCR reaction containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, and 200 μM dNTP. Five cycles were performed (30 sec at 94°C, 30 sec at 48°C, 30 sec at 72°C). Aliquots of 15 μL of products from each reaction were analyzed directly on 1.5% agarose gel with 0.5 μg/mL ethidium bromide.

Sequencing of Amplified Products of Genomic DNA

Five microliters of first-round PCR products were used for sequencing by using the Sequenase PCR Prod-

TABLE I. Sequence and Location of IDS Primers*

Primer	Sequence	Genomic position	cDNA position	Direction
IDS1	5′-TACAGAGTTTTAATTATGGG-3′	Intron 3		Forward
IDS2R	5′-CACAACTTCGTGGTATATAA-3′	Intron 4		Reverse
IDS3C	5′-GGATATCTTCTAACCATACC-3′	Exon 4		Forward
IDS4T	5′-GGATATCTTCTAACCATACT-3′	Exon 4		Forward
IDS5R	5′CATCTTTTCCAACAACTGTATG-3′		615–636	Reverse
IDS7	5′-ACTGGCAGGAGACCTGACAC-3′		277–296	Forward
IDS8	5′-CCTGTACGACTTCAACTCCTAC-3′		303–324	Forward
IDS9C	5′-TATGGGATATCTTCTAACCATACC-3′		417–438	Forward
IDS10T	5′-TGGGATATCTTCTAACCATACT-3′		417–438	Forward
IDS11	5′-GATGATTCTCCGTATAGCTGG-3′		439–459	

*The cDNA is numbered beginning from the ATG of the cDNA coding sequence. A 5′ TA nonspecific tag was added to IDS9C to permit discrimination between the products when separated on a polyacrylamide denaturing gel.

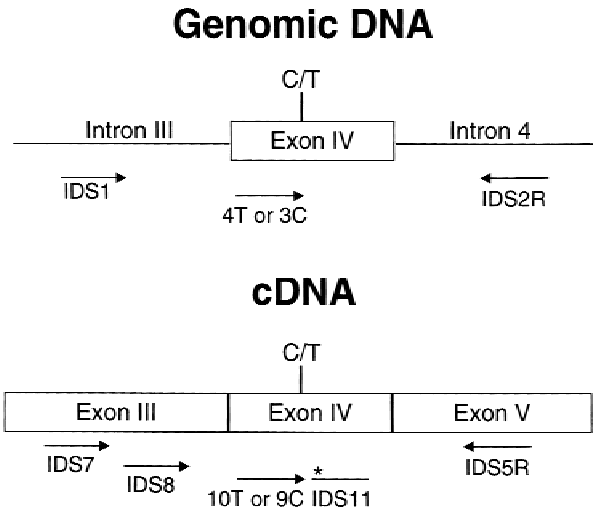

Fig. 3. Location of primers. The asterisk indicates location of ^{32}P.

uct Sequencing Kit (USB, Cleveland, OH), according to the manufacturer's protocol. The sequencing was performed by analyzing both the coding strand (using forward primers) and the complementary strand (using reverse primers). The reaction products were analyzed on a 6% denaturing polyacrylamide gel containing 7 M urea in an 1× glycerol tolerant gel buffer (USB, Cleveland, OH). Gels were fixed in a solution of 15% methanol/5% acetic acid for 15 min and dried at 80°C for 40 min before autoradiography.

Analysis of cDNA for Clonality Determination by ASPCR

Amplification of cDNA containing the IDS polymorphic site was performed using forward primer IDS7 and reverse primer IDS5R. Fifteen pmol of each primer and 1 unit of Taq DNA polymerase were added to a 50-μL PCR reaction (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2 mM $MgCl_2$, 500 μM dNTP). Thirty-five cycles were performed (30 sec at 94°C, 30 sec at 54°C, 30 sec at 72°C). Aliquots of 10 μL of products from each reaction were analyzed directly on a 1% agarose gel with 0.5 μg/mL ethidium bromide, but as this reaction did not always produce visible bands, a second, semi-nested, round of PCR was performed, using the same reverse primer IDS5R and forward primer IDS8. A 1-μL aliquot of first-round products, 15 mM of each primer, and 1 unit of Taq polymerase were added to a 50 μL reaction (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2 mM $MgCl_2$, 500 μM dNTP). Amplification was carried out for 30 cycles (30 sec at 94°C; 30 sec at 57°C; 30 sec at 72°C). Allele-specific PCR was performed in a 50-μL reaction volume containing 5 μL of first- or second-round PCR products, 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTP, 1 unit of Taq polymerase, 15 pM reverse primer 5R, and 15 pM of an allele-specific primer, either IDS9C or IDS10T. Seven cycles were carried out (30 sec at 94°C; 30 sec at 61°C; 30 sec at 72°C). A 15-μL aliquot of products was analyzed on a 1.5% agarose gel with 0.5 μg/mL ethidium bromide.

Detection of Polymorphisms by LDR

The LDR assay for the IDS polymorphism was performed as described (33,34) with modifications. The common ligation partner IDS11 was labeled with [γ-^{32}P] (ICN Pharmaceuticals, Irvine, CA) at the 5′ end by T4 polynucleotide kinase (USB, Cleveland, OH) using the manufacturer's recommended conditions. Cloned thermostable ligase (a kind gift of Dr. Barany, Cornell University Medical College, NY) was diluted in buffer containing 10 mM Tris-HCl, pH 8.0, 50% glycerol, 0.5 mM EDTA, 1 mM dithiothreitol, 100 μg bovine serum albumin, and 0.1% Triton X-100. In the LDR reaction, 4 μL of the first-round PCR reaction was used as template in a 10-μL reaction volume (20 mM Tris-HCl, pH 7.6, 100 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM NAD+, 10

TABLE II. Frequency of IDS Polymorphism

IDS genotype	Caucasian (n = 82)	African-American (n = 31)	Oriental (n = 34)
C	32 (39%)	16 (52%)	34 (100%)
T	12 (15%)	3 (10%)	0 (0%)
TC	38 (46%)	12 (39%)	0 (0%)

mM dithiothreitol) with 20 units of cloned thermostable ligase, 360 μM each of allele specific primers IDS9C and IDS10T, and 180 μM of the adjacent common ligation partner. Denaturing for 1 min at 94°C and ligation for 4 min at 67°C were performed for 30 cycles, using a Thermocycler 480 (Perkin-Elmer, Emeryville, CA). The samples were denatured for 5 min at 90°C, and the electrophoresis was performed in 15% denaturing polyacrylamide gel (acrylamidebisacrylamide ratio, 29:1) in 1× Tris–borate–EDTA buffer. The gels were run for 4.5 hr at constant 5 W power using 17 × 15 cm gels in a V16 vertical electrophoresis apparatus (Life Technologies, Grand Island, NY). After fixation and drying of the gels, the ligation products were detected by autoradiography. The C allele was detected as a 45-nucleotide product, and the T allele was detected as a 43-nucleotide product. The LDR assay for the p55 polymorphism was performed as previously described [21].

Calculations of Gene Allele Frequencies and Linkage Disequilibrium

Maximum likelihood methods [35] were used to calculate the gene frequencies of the different alleles. To test for significant deviations from linkage equilibrium ($D = 0$), a Chi-square test was applied to the male haplotype data [36–38].

RESULTS

Frequency of the IDS Polymorphism

Using ASPCR, the IDS genotype of 147 females (82 Caucasians, 31 African-Americans, and 34 Orientals) was determined (Table II). The Oriental females consisted of 31 Chinese, 1 Vietnamese, 1 Korean, and 1 Thai. Representative ASPCR results are shown in Figure 4. The genotypes obtained by ASPCR were confirmed by sequencing in two cases (data not shown). The sample with a homozygous C genotype by ASPCR had a C nucleotide while the sample with a homozygous T genotype had a T nucleotide at the same position. Forty-six percent of 82 Caucasian females and 39% of 31 African-American females were heterozygous for the polymorphism and thus informative for clonality studies. In contrast, all 34 Oriental females examined were homozygous for the C allele.

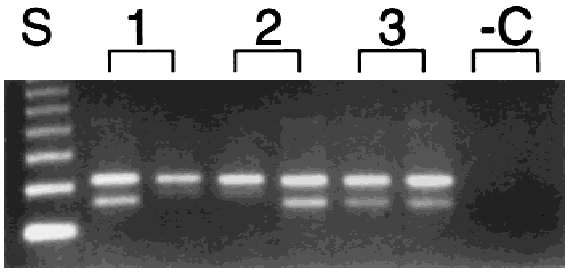

Fig. 4. Determination of IDS genotype. Firstround PCR products are subjected to allelespecific PCR reactions. The first well contains the products primed with the T-specific oligonucleotide; the second well contains the products primed with the C-specific oligonucleotide. S = molecular weight standard. Sample 1 is homozygous for the T allele. Sample 2 is homozygous for the C allele. Sample 3 is heterozygous for the polymorphism. −C = negative control (simultaneously processed sample with substitution of H_2O for DNA).

Proportion of Females Informative for Clonality Studies

The frequencies of the G6PD, p55 and IDS polymorphisms were determined in 70 Caucasian and 29 African-American females. Fifty-three percent of Caucasian and 48% of African-American females were heterozygous at either the G6PD or the p55 locus. When the IDS polymorphism was included, the percentage of females heterozygous at at least one of the loci increased to 76% in Caucasians and 62% in African-Americans.

Linkage Analysis

The G6PD, p55, and IDS genes are all located on Xq28 [28,29,39,40]. Analysis of linkage between the G6PD and p55 genes was previously reported [22]. To establish the presence of any possible linkage between the IDS gene and either the G6PD or the p55 gene, the genotype of 103 Caucasian and 62 African-American males was determined. The allelic frequencies are shown in Table III. Analysis for linkage disequilibrium showed that G6PD and IDS are in linkage equilibrium in both Caucasians ($D = 0.0059$, $\chi^2 = 0.161$, $P > 0.05$) and African Americans ($D = 0.0094$, $\chi^2 = 0.224$, $P > 0.05$). p55 and IDS are also in linkage equilibrium ($D = -0.0373$, $\chi^2 = 2.440$, $P > 0.05$ for Caucasians; $D = -0.0187$, $\chi^2 = 0.549$, $P > 0.05$ for African-Americans).

Expression of the IDS Gene

The clonality assays based on transcriptional polymorphisms of the X-linked, ubiquitously expressed G6PD and p55 genes are useful for studying all hematopoietic cells, including the non-nucleated platelets and reticulocytes. Because IDS encodes a lysosomal enzyme, it was unknown if it was expressed in all cell lineages. ASPCR analysis of cDNA demonstrated transcripts of the IDS

TABLE III. Allelic Frequencies in Males

Allele	Caucasians	African-Americans
IDS - C	0.68	0.76
IDS - T	0.32	0.24
p55 - T	0.65	0.32
p55 - G	0.35	0.68
G6PD - C	0.88	0.84
G6PD - T	0.12	0.16

alleles in all hematopoietic cells including reticulocytes, which do not contain lysosomes (Fig. 5).

IDS Gene Is Subject to X Inactivation

When one of the X chromosomes is inactivated in embryogenesis, a few genes escape inactivation [41]. To be useful for clonality studies, the IDS gene must be subject to X inactivation so that only one allele will be detected in a monoclonal cell population.

The myeloproliferative disorders chronic myelogenous leukemia, polycythemia vera, and essential thrombocythemia are characterized by clonal hematopoiesis of the myeloid cells [8,42,43]. We previously analyzed cells from patients with myeloproliferative disorders for clonality using rtPCR-LDR to detect polymorphisms of the G6PD and p55 genes [44]. To demonstrate that the IDS gene is inactivated, we tested the cDNA from myeloid cells from four patients who were heterozygous for the IDS polymorphism. One patient with essential thrombocythemia was previously determined to have monoclonal platelets, neutrophils, and reticulocytes by transcriptional analysis for the p55 polymorphism (Fig. 6A). ASPCR analysis of the cDNA obtained from her platelets, neutrophils, and reticulocytes for the IDS polymorphism detected only transcripts of the T allele (Fig. 6B), confirming that the IDS gene is subject to inactivation. Identical results were obtained from LDR analysis (data not shown). Neutrophils and platelets from two patients with polycythemia vera also expressed only one allele, but neutrophils and platelets from a patient with reactive thrombocytosis expressed both alleles, indicating polyclonal hematopoiesis (data not shown).

DISCUSSION

We report a simple assay for an exonic polymorphism of the IDS gene that increases the number of females suitable for clonality analysis using transcriptional assays. The non-radioactive PCR-based assays permit screening for genotype and rapid determination of clonality. When desired, the rtPCR-LDR assay can be used to quantitate the ratio of allelic transcripts in polyclonal populations. El Kassar et al. [45] also used the IDS polymorphism to study clonality in a population of

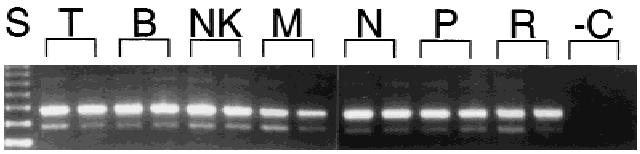

Fig. 5. Expression of the IDS gene. Transcripts of the IDS gene are detectable in all hematopoietic cell lineages. mRNA from peripheral blood cells from an IDS heterozygous female was transcribed to cDNA, the polymorphic region was amplified by PCR and then subjected to allele specific PCR reaction. S = molecular weight standard. T = T lymphocytes; B = B lymphocytes; NK = NK cells; M = monocytes; N = neutrophils; P = platelets; R = reticulocytes; −C = negative control (simultaneously processed sample with substitution of H_2O for mRNA).

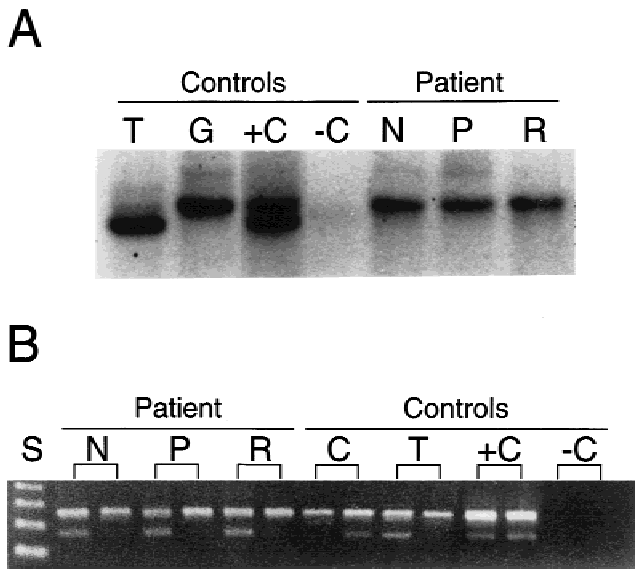

Fig. 6. Clonality analysis. cDNA obtained from the neutrophils, platelets, and reticulocytes of a patient with essential thrombocythemia who was heterozygous for both the p55 and IDS polymorphisms was analyzed for expression of the p55 and IDS alleles. (A) LDR analysis for transcripts of the p55 gene. Controls: T = homozygous for T allele; G = homozygous for G allele; +C = positive control (normal subject heterozygous for T and C alleles); −C = negative control (simultaneously processed sample with substitution of H_2O for mRNA). Patient: N = neutrophils; P = platelets; R = reticulocytes. (B) ASPCR analysis for transcripts of IDS gene. S = molecular weight standard. Patient: N = neutrophils; P = platelets; R = reticulocytes. Controls: C = homozygous for C allele; T = homozygous for T allele; +C = positive control (normal subject heterozygous for C and T alleles); −C = negative control (simultaneously processed sample with substitution of H_2O for mRNA).

French females with thrombocytosis but employed a method based on restriction enzymes for detecting the polymorphism.

The IDS locus is the most informative of the polymorphisms studied to date and combining this assay for the IDS C/T polymorphism with our previously developed

assays for the G6PD and p55 polymorphisms increased the number of heterozygous (informative) Caucasian females from 53% to 76% and the number of informative African-American females from 48% to 62%. Interestingly, the IDS C/T polymorphism was not detected in Oriental females, suggesting that it arose from a wild-type C allele after the races diverged. On a practical level, the absence of the polymorphism in Orientals makes screening of the IDS genotype in Oriental females unproductive as it will not increase the number of informative females. While at the IDS and G6PD loci, the same (wild-type) allele predominates in all racial groups, at the p55 locus the T allele predominates in Caucasians and the G allele predominates in African-Americans. Linkage disequilibrium analysis confirmed that the IDS gene is not linked to either the G6PD or the p55 gene; thus, the presence of the IDS polymorphism is independent of the alleles present at either the G6PD or the p55 locus.

The ideal clonality assay would be applicable not only to all females but also to males. However, with the exception of disease-specific clonal markers such as the Philadelphia chromosome in CML or clonal immunoglobin gene rearrangement and its products in B cell malignancies, no such an assay or method is available. The X-chromosome inactivation assays based on differential DNA methylation of active and inactive chromosomes, while useful only in females, are informative for more than 95% of all females. Although the clonality assays based on differentiation of active and inactive chromosomes by detection of transcripts are informative in a lower proportion of females (~75%), they hold several important advantages. Unlike methylation-based assays, transcriptional assays are quantitative, applicable to nonnucleated cells (such as platelets and reticulocytes) and are biologically more sound, thus providing in certain situations more accurate information. For example, we recently had the opportunity to study cultured neuroblastoma cell lines from females that appeared inexplicably polyclonal by methylation assay but which were clonal by the IDS transcriptional assay (Gillian, Maris, Kralovics, and Prchal, in preparation), suggesting that DNA methylation can change under different culture conditions or in malignant tissues. Similar discrepancies were also reported by El Kassar et al. [45] in their studies of essential thrombocythemia.

Clonality assays based on the detection of exonic or transcriptional polymorphisms of X-linked genes represent an improvement over previous clonality assays. One limitation of these assays, however, is the few number of exonic polymorphisms relative to intronic polymorphisms. The IDS transcriptional assay increases the number of informative females from ~50% [22] to ~75%; identification of exonic polymorphisms in other X-chromosome genes and developing assays to detect them would increase the number of informative females. Ultimately, virtually all females should be suitable for clonality analysis.

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